Ephemeroptera Larvae in Singapore: a Taxonomical Approach

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ABSTRACT

Pollution-sensitive mayflies have been used extensively in water quality monitoring studies. In order to realise more of their ecological importance, it is important to be able to describe and understand the ecology of that taxa. Ephemeroptera in Singapore have not been extensively described so far. This study aimed to establish the baseline for identifying local ephemeropteran taxa in lentic habitats and a key was generated based on specimens from Lower Peirce reservoir. A total of 3 families, 4 genera and 1 subgenus were recorded. An alternative taxonomical approach was tested, using DNA barcoding methods. This provided ample evidence to distinguish between specimens but more work is needed to verify species-wise distinction. Methods were devised to rear live nymphs for larval-adult matching. These methods include transportation of live nymphs and the designing of a rearing chamber which managed to sustain 10 nymphs for periods ranging from 1 to 8 days. This study is a significant step in establishing preliminary information on ephemeropteran taxa in Singapore.

INTRODUCTION

Studies involving Ephemeroptera in the Southeast Asian region have not included Singapore extensively thus far. As knowledge of mayflies in Singapore is still preliminary, this study will aim to achieve three main objectives. First, to document the Ephemeroptera identities in local lentic habitats, whilst contributing to the generation of a pioneering taxonomic key for Singapore’s Ephemeroptera nymphs with the help of the Lucid3 system. Second, to test the ability of DNA sequencing methods to correctly identify mayfly larvae. Third, to design an experimental setup to sustain live nymphs to match them to their adult forms for a more comprehensive identification approach, and to observe their ecology in the rearing chamber.

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MATERIALS AND METHODS

Morphology

Ephemeropteran nymphs from Lower Peirce reservoir on 4 April 2008 were identified using mainly Asian taxonomic keys such as Dudgeon (1999) and Yule and Yong (2004). Mounting of nymphs and key features for that particular taxa onto glass slides using fine tipped insect pins with glycerine, and an Olympus 265212 microscope of up to 400x magnification facilitated identification greatly.

DNA Barcoding for Biological Identification

In order to lend support to morphological identification of several ephemeropteran larvae, I approach this from a genetic perspective. A 650-base-pair fragment of the gene cytochrome c oxidase 1 (COI) has been used reliably to separate mayfly species in DNA barcoding (Sta˚hls and Savolainen, 2008). Here, methods of DNA extraction as well as the ability of DNA barcoding to correctly identify several mayfly larvae was tested. Freshly killed nymphs from Lower Peirce reservoir were preserved in 100% ethanol at -70° to prevent DNA degradation. 3 Povilla and 1 Caenidae specimen was identified morphologically at the genus and family level respectively and used as mock unknowns.

Sustenance of Mayfly Nymphs in a Simple System

Accurate identification of species requires the close inspection of all life stages for most aquatic insects. However, identification has been mostly based on either the nymphs or adults only with no association being made (Merritt et al., 1996), leading to possible misidentification. A rearing setup was designed to sustain a total of 10 mayflies caught in Lower Peirce reservoir in February 2009 using traps made from coconut brushes and wooden sticks. Morphological variation was noted before placing nymphs into the rearing chamber and their behaviour was observed frequently.

RESULTS

The sampling of nymphs from one of the stations in Lower Peirce reservoir appeared to sustain a total of 3 families, 4 genera and 1 subgenus. They are as follows: family Polymitarcyidae, genus Povilla and subgenus Povilla; family Caenidae and genus Caenodes; family Baetidae and genera Baetis and Cloeon. Drawings in the key to Ephemeroptera larvae are included. Fig. 1 depicts a general Ephemeroptera larvae categorised by three tails and abdominal gills.
Genetic differences in the COI gene sequences amongst 4 ephemeropteran nymphs are summarised in Table 1.

### Table 1 Genetic differences (%) between pairs of COI gene sequences.

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Caenidae sp.</th>
<th>Povilla specimen 1</th>
<th>Povilla specimen 2</th>
<th>Povilla specimen 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caenidae sp.</td>
<td>—</td>
<td>17.75</td>
<td>17.99</td>
<td>18.13</td>
</tr>
<tr>
<td>Povilla specimen 1</td>
<td>17.75</td>
<td>—</td>
<td>0.17</td>
<td>0.00</td>
</tr>
<tr>
<td>Povilla specimen 2</td>
<td>17.99</td>
<td>0.17</td>
<td>—</td>
<td>0.16</td>
</tr>
<tr>
<td>Povilla specimen 3</td>
<td>18.13</td>
<td>0.00</td>
<td>0.16</td>
<td>—</td>
</tr>
</tbody>
</table>

The closest match of the COI genes sequenced from the 4 individuals against all GenBank sequences using BLAST are summarised in Table 2.

### Table 2 Closest genetic match of sequences from GenBank

<table>
<thead>
<tr>
<th>Family</th>
<th>Specimen name</th>
<th>Closest match in GenBank</th>
<th>Similarity (%)</th>
<th>Gene accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caenidae</td>
<td>Caenidae sp.</td>
<td>Caenis punctata</td>
<td>84</td>
<td>AY326825.1</td>
</tr>
<tr>
<td>Polymitarcyida</td>
<td>Povilla specimen 1</td>
<td>Lucilia sericata</td>
<td>83</td>
<td>AJ417716.1</td>
</tr>
<tr>
<td>Polymitarcyida</td>
<td>Povilla specimen 2</td>
<td>Lucilia sericata</td>
<td>83</td>
<td>AJ417716.1</td>
</tr>
<tr>
<td>Polymitarcyida</td>
<td>Povilla specimen 3</td>
<td>Lucilia sericata</td>
<td>83</td>
<td>AJ417716.1</td>
</tr>
</tbody>
</table>

Fig. 1 Dorsal view of a general Ephemeroptera larvae
Methods devised to sustain live larvae while transportation resulted in high mortality and the rearing chamber managed to sustain ten of the successfully transported nymphs of various instars for 1 to 8 days. Their duration of survival was too short for any noticeable morphological change to be seen, and no exuviae was found within this period. Behaviour of Polymitarcyidae and Caenidae nymphs was consistent with that of similar studies under laboratory conditions.

DISCUSSION

The generated key can be generally used to separate nymphs of three mayfly families and four genera in Singapore based on taxa from Lower Peirce reservoir but needs expansion and verification by experts. It is shown from Table 1 that the COI gene can separate families distinctively although more comparisons are needed for species-wise differentiation. The genetic information in GenBank does not cover all Ephemeroptera families extensively and is important for having a baseline comparison. The rearing chamber did not manage to sustain any nymphs to imago stage, possibly due to insufficient oxygen with regards to the different oxygen requirements for each family of Ephemeroptera. Nevertheless, such studies are feasible with enough time and clearly in need to reconcile nymphs with adults during situations where uncertainty concerning characteristic features unique to developmental stages arises. This study is a significant step in establishing preliminary information on ephemeropteran taxa in Singapore.

REFERENCES